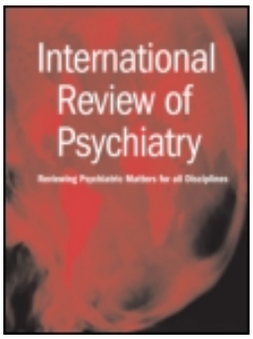

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Basic neuroanatomy and neuropharmacology of cannabinoids

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Abstract
Humans have used *Cannabis sativa* (marijuana) for at least 12,000 years, but researchers have only recently described an endogenous cannabinoid system. The endocannabinoid system modulates an array of physiological and psychological functions. Endocannabinoids are widely distributed throughout the body, including the central nervous system (CNS). This article gives a basic overview of endocannabinoid neuroanatomy and function. Several endocannabinoids have been discovered to date, and their roles are being elucidated. Two G-protein coupled cannabinoid receptors, CB1R and CB2R, have been identified, although other candidate receptors exist, including ion channel and nuclear receptors that might be components of the endocannabinoid system. It appears that cannabinoids are dysregulated in a number of psychiatric disorders and might be involved in their pathogenesis. There is now evidence that manipulation of the endocannabinoid system could be a therapeutic target for a variety of conditions.

Introduction

Ingestion of cannabis produces effects including changes in mood, sedation, altered perception, increased appetite, decreased nausea, and impairment of memory, executive function and coordination. Physiological effects include analgesia, neuroprotection, and decreased intraocular pressure, body temperature, inflammation and neuronal excitability. Delta-9-tetrahydrocannabinol (THC) is the primary active component of marijuana (Gaoni & Mechoulam, 1964), though other constituents, like cannabidiol (CBD), have biological effects. The discovery of cannabinoid binding sites in brain (Devane et al. 1988) revealed an endocannabinoid system and provided the basis for understanding cannabinoid pharmacology. Recent evidence suggests that the endocannabinoid system also modulates reinforcement/addiction, and may be involved in a number of neurological conditions (reviewed in this issue).

The CNS endocannabinoid system will be reviewed, with consideration of the following:

- Cannabinoids are localized both in regard to cellular and neuroanatomical circuits to modulate mood, reward, cognition and perception, as may be related to psychiatric disorders.
- Elucidation of the function and localization of endocannabinoids, their receptors and their synthetic and degradative enzymes will clarify the role of the endocannabinoid system in psychiatric disorders and expand their therapeutic potential.

Overview of the CNS endocannabinoid system

Cannabinoid receptors were first demonstrated by pertussis toxin-sensitive inhibition of cAMP formation (Howlett, 1984) and confirmed by cloning of CB1R from brain (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). CB2R were subsequently cloned from immune tissue (Munro, Thomas, & Abu-Shaar, 1993). Autoradiography of cannabinoid binding (Glass, Dragunow, & Faull, 1997; Herkenham et al., 1991) or mRNA (Matsuda et al., 1990) revealed high receptor density with widespread distribution in brains of multiple species, including areas regulating motor activity, memory, pain, homeostasis and reward. Structure-activity studies indicated correlation between CB1R agonist affinity and potency in behavioural/physiological measures (Compton et al., 1993; Herkenham et al., 1990). Studies in mice lacking CB1R confirmed that CB1R mediates most CNS effects of THC (Ledent et al., 1999; Zimmer, Zimmer, Hohmann, Herkenham, & Bonner, 1999). Recent evidence suggests additional CNS sites of action, including G-protein-coupled receptors (GPCRs), ion channels and nuclear receptors (see Future Directions).

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Several endocannabinoids have been identified, and the synthesis and degradation of two have been elucidated. Arachidonoyl-ethanolamine (anandamide) and 2-arachidonoyl-glycerol (2-AG) are the best characterized, whereas 2-arachidonyl-glyceryl ether (noladin), *O*-arachidonoyl-ethanolamine (virhodamine) and *N*-arachidonoyl-dopamine (NADA) are more recent discoveries (Piomelli, 2003). Anandamide and 2-AG activate CB1R and CB2R, as well as novel cannabinoid binding sites (see Future Directions). Recent evidence suggests that anandamide is synthesized by the previously uncharacterized α/β-hydrolase-4 and glycerophosphodiesterase GDE1 (Simon & Cravatt, 2008). Anandamide is degraded by fatty acid amide hydrolase (FAAH), which also metabolizes other fatty acid amides. 2-AG is produced by phospholipase C and diacylglycerol lipase and is degraded by monoacylglycerol lipase (Ahn, McKinney, & Cravatt, 2008). Complete identification of the synthesis and degradation pathways for all will provide novel targets for drugs to manipulate brain endocannabinoid levels. Elucidation of the relationship between anandamide and 2-AG in endocannabinoid function will clarify the role of this system in psychiatric disorders.

Synthetic cannabinoid ligands have been developed and are used primarily for research purposes. Nantradol, levonantradol and HU-210 are similar structural analogues of THC, a tricyclic dibenzopyran. CP55940 is a bicyclic analogue lacking the pyran ring, whereas WIN55212-2 is an aminoalkylindole (Howlett et al., 2004) widely used *in vitro* because of its high efficacy (Breivogel, Selley, & Childers, 1998). Interestingly, these synthetic agonists and 2-AG exhibit higher efficacy at CB1R and CB2R than THC, a low efficacy partial agonist (Sim, Hampson, Deadwyler, & Childers, 1996), or anandamide, an intermediate-efficacy partial agonist (Breivogel et al., 1998). Specific antagonists of CB1R (e.g. SR141716A) and CB2R (e.g. SR144528) have also been developed.

Cannabinoid receptor signalling occurs via a variety of intracellular pathways. Cannabinoids have predominantly inhibitory actions, by decreasing release of neurotransmitters (Breivogel, Walker, Huang, Roy, & Childers, 2004; Svizenska, Dubovy, & Sulcova, 2008), and hormones (Di, Malcher-Lopes, Halmos, & Tasker, 2003). Anatomical studies using single-cell resolution mRNA labelling (Marsicano & Lutz, 1999) or conditional mutant mice (Monory et al., 2007) support CB1R modulation of both excitatory and inhibitory neurotransmission because CB1R are located on both GABAergic and principal (glutamatergic) neurons. Endocannabinoids can be released in an activity-dependent manner by postsynaptic neurons and bind to presynaptic receptors to inhibit neurotransmitter release, a process termed retrograde signalling (Kreitzer & Regehr, 2001; Wilson & Nicoll, 2001). At the cellular level, CB1R activate primarily $G\alpha_i$ and $G\alpha_o$ (Glass & Northup, 1999; Prather, Martin, Breivogel, & Childers, 2000) to inhibit adenylyl cyclase (AC), activate G-protein-gated inwardly rectifying K^+ (GIRK) channels, inhibit P, Q and N-type Ca^{2+} channels, and activate mitogen-activated protein kinase (MAPK). CB2R only activate $G\alpha_i$ subtypes to inhibit AC and activate MAPK (Howlett et al., 2004). Moreover, CB1R form homo-oligomers (Mackie, 2005; Wager-Miller, Westenbroek, & Mackie, 2002) and heterodimers with dopamine D2 (Kearn, Blake-Palmer, Daniel, Mackie, & Glass, 2005) and mu opioid (Rios, Gomes, & Devi, 2006) receptors, which could activate alternative pathways. While the *in vivo* implications are unclear, co-stimulation of CB1R and D2R, for example, can stimulate AC (Glass & Felder, 1997; Kearn et al., 2005).

Cannabinoid therapeutic uses

Synthetic THC (dronabinol/Marinol®) is approved in North America for antiemesis in chemotherapy and appetite stimulation in HIV/AIDS. Clinical or anecdotal evidence suggests effectiveness of cannabis or CB1R agonists in depression, anxiety disorders, attention deficit disorder, and Tourette's syndrome, and several non-psychiatric disorders. Widespread clinical use is complicated by CB1R-mediated psychotropic effects and the potential for dependence (Kogan & Mechoulam, 2007). Cannabidiol (CBD) might also have beneficial effects for neuronal diseases or injury. An advantage of CBD is that it does not activate CB1R and might counteract some psychotropic effects induced by CB1R (Morgan & Curran, 2008). Sativex®, an extract of cannabis containing THC and CBD, was developed based on the hypothesis that additional therapeutic effects arise from the combination of compounds in cannabis compared to THC alone. Clinical studies indicate efficacy of Sativex® for a number of disorders (Wright, 2007). It is already approved in Canada to treat pain, and is in clinical trials in the US and Europe. CB1R antagonists (e.g. rimonabant) show promise as pharmacotherapeutics for obesity and addiction, but most clinical trials were terminated in 2008 over concerns of depression/suicide.

CNS distribution of CB1 receptors and endocannabinoids

The distribution of cannabinoid receptors is similar in primate and rodent brain (Glass et al., 1997; Herkenham et al., 1991). Receptor levels in humans are highest in the hippocampal complex, entorhinal

and cingulate cortices, frontal gyrus, amygdaloid complex, substantia nigra and cerebellar molecular layer. Levels are low in thalamic and hypothalamic nuclei, brainstem (except dorsal motor nucleus of the Vagus) and spinal cord. Relative receptor levels are highest in associative regions of frontal and limbic cortices, and low to moderate in primary and secondary motor and sensory cortices, respectively. Interestingly, receptor levels are higher in the left (dominant) cerebral hemisphere associated with verbal communication. Autoradiography in rodent brain (Herkenham et al., 1991; Jansen, Haycock, Ward, & Seybold, 1992) revealed a higher level of receptors in basal ganglia and a less complex cortical distribution than in humans. The distribution of CB1R mRNA is similar to CB1R, with high levels in human forebrain and cerebellar cortex (Wang, Dow-Edwards, Keller, & Hurd, 2003). A similar distribution occurs in foetal and neonatal brain, but CB1R and mRNA were higher in basal ganglia and the limbic system compared to adult brain (Glass et al., 1997; Wang et al., 2003). CB1R mRNA was absent from cerebellum, probably because of delayed neurogenesis. These findings might be relevant to reports that prenatal exposure to heavy cannabis use produces lasting behavioural/cognitive effects (Fried & Smith, 2001).

The subcellular localization of CB1R has been elucidated utilizing antibodies to CB1R (Egertova & Elphick, 2000; Tsou, Brown, Sanudo-Pena, Mackie, & Walker, 1998). CB1R-immunoreactivity (CB1R-ir) was found predominantly in fibres, with similar distribution and density as demonstrated by autoradiography. In addition, CB1R-ir cells were identified in the forebrain and cerebellum, though principal (pyramidal and Purkinje) neurons were immunonegative (Tsou et al., 1998). In monkey, CB1R-ir was high in cerebral cortex, hippocampus, amygdala and cerebellum, but principal cells were immunopositive and receptor levels were lower in the output nuclei of the basal ganglia (Ong & Mackie, 1999). Electron microscopy revealed that CB1R-ir was localized in both dendritic spines and axon terminals, indicating pre- and post-synaptic localization.

Comparison of CB1R levels with cannabinoid effects reveals some apparent mismatches. For example, cannabinoids modulate homeostasis (Breivogel & Childers, 1998), but CB1R levels in hypothalamus are low. Examination of G-protein activity via cannabinoid-stimulated [^{35}S]GTPγS autoradiography (Sim, Selley, & Childers, 1995) revealed a similar distribution to CB1R, but found regional differences in the relative levels of CB1R and CB1R-activated G-proteins (Sim et al., 1995). CB1R efficiency (number of G-proteins activated per CB1R) is highest in hypothalamus (Breivogel, Sim, & Childers, 1997), implying that stimulation of fewer CB1R receptors is sufficient to achieve biological effects. Furthermore, efficiency varies within regions because G-protein activation in the anterior cingulate cortex was higher in lamina VI than in lamina I, despite equivalent receptor levels (Sim-Selley, Vogt, Vogt, & Childers, 2002). The levels of CB1R and CB1R-activated G-proteins can change during cannabinoid treatment. Chronic administration to rodents produces CB1R desensitization and down-regulation in regions that mediate physiological and motivational effects (Sim-Selley, 2003). Relevance to human physiology is supported by decreased CB1R binding in the hippocampus, striatum/basal ganglia and ventral mesencephalon of regular marijuana users (Villares, 2007).

Antibodies binding to endocannabinoid synthetic and degradative enzymes have used as a proxy for the localization of the endocannabinoids themselves. Immunoreactivity of the anandamide degrading enzyme, FAAH, was most prominent in pyramidal neurons of cerebral cortex and hippocampus, and Purkinje neurons of cerebellum in rats (Egertova, Giang, Cravatt, & Elphick, 1998; Gulyas et al., 2004) and humans (Romero, Hillard, Calero, & Rabano, 2002). Moderate to lightly stained FAAH-ir neurons were more widely distributed. Comparison of FAAH-ir and CB1R-ir (Egertova, Cravatt, & Elphick, 2003) showed that FAAH-ir soma were surrounded by CB1R-ir fibres in certain regions (amygdala, hippocampus, neocortex, cerebellum), whereas other areas exhibited a potential mismatch. This suggests the existence of additional receptors or endocannabinoids. Immunoreactivity of the 2-AG synthesizing enzyme, diacylglycerol lipase α, was found post-synaptically on dendritic spines of Purkinje and hippocampal pyramidal cells (Katona et al., 2006; Yoshida et al., 2006), consistent with a role for 2-AG in retrograde signalling (Yoshida et al., 2006). The idea that 2-AG predominates in retrograde signalling (Gulyas et al., 2004; Jonsson, Holt, & Fowler, 2006; Melis et al., 2004) is supported by the finding of presynaptic FAAH-ir and postsynaptic monoacylglycerol lipase-ir (the 2-AG degrading enzyme) (Gulyas et al., 2004).

Anatomical substrates of cannabinoid-mediated effects

Physiological effects

In vivo testing of cannabinoids often involves assessing the tetrad of hypolocomotion, catalepsy, hypothermia, and antinociception (Compton et al., 1993). Studies in engineered mice lacking CB1R in either principal/glutamate or GABAergic neurons indicated that these effects are mediated by CB1R

on glutamatergic neurons (Monory et al., 2007). Effects on motor function are likely mediated by CB1R in motor cortex, basal ganglia and cerebellum (Breivogel & Childers, 1998), and ataxia/incoordination via CB1R in cerebellum (DeSanty & Dar, 2001; Patel & Hillard, 2001). The external segment of the globus pallidus (Pertwee & Wickens, 1991), striatal medium spiny neurons and cortical glutamatergic cells (Monory et al., 2007) are implicated in catalepsy. Cannabinoids produce hypothermia when injected into the preoptic area of hypothalamus (Fitton & Pertwee, 1982; Rawls, Cabassa, Geller, & Adler, 2002). Cannabinoids mediate antinociception by spinal and supraspinal mechanisms (Lichtman & Martin, 1991). The PAG and rostral ventral medulla (RVM) are components of a descending analgesic pathway to the dorsal horn of the spinal cord (Fields, Heinricher, & Mason, 1991). Direct administration of cannabinoids into the ventrolateral PAG (Martin, Patrick, Coffin, Tsou, & Walker, 1995) or RVM (Martin, Tsou, & Walker, 1998) produces antinociception. CB1R act in the RVM and PAG by presynaptic inhibition of GABA transmission, disinhibiting antinociceptive neurons (Meng, Manning, Martin, & Fields, 1998). This mechanism is similar, but not identical, to that of brainstem-mediated antinociception by opioids. Cannabinoids also modulate nociception via the ventroposterolateral (VPL) nucleus of the thalamus (Martin, Hohmann, & Walker, 1996). Interestingly, administration of a CB2 agonist into the VPL produces antinociception in a rodent model of neuropathic pain (Jhaveri et al., 2008), suggesting multiple cannabinoid mechanisms. CB1R in the spinal cord are highest in superficial laminae (I and II) and lamina X (Farquhar-Smith et al., 2000), and dorsal root ganglion (Ahluwalia, Urban, Capogna, Bevan, & Nagy, 2000). CB1R are also on peripheral nociceptors, and mediate inhibition of inflammatory and neuropathic pain (Agarwal et al., 2007).

Abuse and dependence

Cannabinoids are widely used recreationally and maintain self-administration in certain animal models (see Cooper and Haney). The mesocorticolimbic system, in which dopaminergic neurons in the ventral tegmental area (VTA) project to regions including nucleus accumbens (NAC) and prefrontal cortex (PFC), appears to contribute to drug reinforcement. Microinjections of cannabinoids into NAC shell or VTA support self-administration (Zangen, Solinas, Ikemoto, Goldberg, & Wise, 2006). Conversely, antagonist-precipitated withdrawal in cannabinoid-tolerant animals reduces dopamine in NAC shell (Tanda, Loddo, & Di Chiara, 1999), and inhibits dopamine cell firing (Diana, Melis, Muntoni, & Gessa, 1998) and brain stimulation reward (Gardner & Vorel, 1998), consistent with withdrawal from other psychoactive drugs.

CB1R have been localized in the mesocorticolimbic system using anatomical and electrophysiological approaches (Lupica, Riegel, & Hoffman, 2004). Dopaminergic neurons in the VTA that project to NAC are modulated by cannabinoids (French, 1997). CB1R are positioned on both GABAergic (from NAC and other regions) and glutamatergic (from cortex) afferent terminals and regulate endocannabinoid release onto VTA dopamine neurons (Lupica et al., 2004). CB1R in NAC are located on both cortical glutamatergic afferents and intrinsic GABAergic terminals. Thus, cannabinoids modulate excitatory and inhibitory activity in both NAC and VTA. CB1R are co-localized with mu opioid and D2R (Pickel, Chan, Kash, Rodriguez, & MacKie, 2004; Pickel, Chan, Kearn, & Mackie, 2006) in NAC, which might modulate reward of cannabinoids and other drugs. In addition, CB1R and D2R exhibit overlapping subcellular distributions in some axons and terminals, providing the possibility for CB1R/D2R heterodimers.

Anxiety and mood

In humans, low dose THC is generally euphoric and anxiolytic, whereas higher doses can be dysphoric and anxiogenic (Hollister, 1986). Withdrawal from cannabinoids produces irritability, anxiety, insomnia and decreased appetite (Haney, Ward, Comer, Foltin, & Fischman, 1999; Jones, Benowitz, & Herning, 1981). Mice lacking CB1R exhibit an anxiogenic and depressed phenotype, and CB1R antagonists can produce anxiety in rodents (Fattore et al., 2007). The relationship between cannabis and depression is complicated, since both chronic consumption and withdrawal are associated with depression. The amygdala is part of the circuit that mediates mood and the acquisition and extinction of fear conditioning. CB1R are on interneurons in the amygdala and act via retrograde signalling to suppress GABA release, functionally disinhibiting neurons (Katona et al., 2001). Recent studies indicate that CB1R activation is necessary for extinction of fear conditioning, and that FAAH inhibitors (that slow anandamide degradation) enhance extinction (Chhatwal & Ressler, 2007). These findings suggest a neural mechanism by which cannabinoids might influence anxiety and mood disorders.

Psychotic disorders

Alterations in cerebral cortex may contribute to schizophrenia, and dopamine receptors in this region

are an important therapeutic target (Lidow, Williams, & Goldman-Rakic, 1998) (see also Andrew et al., pp. 152–167 in this issue). The role of cortical CB1R in schizophrenia is suggested by reports that CB1R are increased in the dorsal lateral prefrontal cortex (Dean, Sundram, Bradbury, Scarr, & Copolov, 2001), anterior cingulate cortex (Zavitsanou, Garrick, & Huang, 2004) and posterior cingulate cortex (Newell, Deng, & Huang, 2006) of schizophrenic subjects, though some studies do not support this link (Eggan, Hashimoto, & Lewis, 2008; Koethe et al., 2007). The distribution of CB1R in human neocortex is heterogeneous with regard to regional and laminar distribution, with CB1R-ir primarily localized to axons (Eggan & Lewis, 2007). The highest levels were found in association areas like prefrontal and cingulate cortices (Eggan & Lewis, 2007; Glass et al., 1997). Interestingly, administration of typical (haloperidol) or atypical (clozapine) antipsychotics produces CB1R desensitization in the prefrontal cortex of female rats (Wiley et al., 2008).

Future directions

The cannabinoid field has advanced rapidly since the identification of cannabinoid receptors and endocannabinoids. However, many questions remain, especially in regard to the role of the endocannabinoid system in psychiatric disorders. Two future directions are considered:

- Glia might be an important locus of endocannabinoid actions in the CNS
  Most studies have focused on neuronal cannabinoid receptors, but actions at glia have also been reported. This is particularly significant because of the recent demonstration of astrocyte regulation of non-synaptic interneuronal communication (Navarrete & Araque, 2008). Both FAAH (Romero et al., 2002) and CB1R (Salio, Doly, Fischer, Franzoni, & Conrath, 2002) are expressed in astrocytes, and astrocytes release anandamide in a calcium-dependent manner in response to physiologically relevant stimuli (Walter, Franklin, Witting, Moller, & Stella, 2002). In hippocampus, astrocyte glutamate release is regulated by endocannabinoids released from neurons, and released glutamate activates N-methyl D-aspartate (NMDA) receptors on pyramidal neurons. The regional extent of this type of interaction is unknown, but provides an anatomical substrate for endocannabinoid-mediated interneuronal communication. Significance in psychiatric disorders is suggested by decreased CB1R-ir in glia of the anterior cingulate cortex of subjects with major depression (Koethe et al., 2007).
- The CNS endocannabinoid system may include additional receptors
  Numerous studies have demonstrated non-CB1R-mediated cannabinoid actions in the CNS (Breivogel, Griffin, Di Marzo, & Martin, 2001; Zimmer et al., 1999). Recently, CB2R mRNA was found in cortex, and CB2R mRNA and immunoreactivity were found in cerebellum and brain stem (Van Sickle et al., 2005). Other studies reported widespread distribution of CB2-ir in hippocampus (Brusco, Tagliaferro, Saez, & Onaivi, 2008), and forebrain (Gong et al., 2006; Onaivi et al., 2006). While CB1R are generally presynaptic, CB2R in neurons appear to be postsynaptic (Brusco et al., 2008). The function of CNS CB2R may include antiemesis (Van Sickle et al., 2005) and antinociception (Sagar et al., 2005). CB2R-selective agonists have an advantage as therapeutics because they lack CB1R-mediated psychoactive effects.

GPR55 is a recently discovered GPCR (Sawzdargo et al., 1999) expressed in many brain areas, spinal cord and DRG neurons (Lauckner et al., 2008; Ryberg et al., 2007). In cultured cells GPR55 is activated by anandamide, 2-AG, noladin, virhodamine, THC, CP55940 and HU210. The anatomical localization and physiological roles of this receptor are yet to be determined.

Several cannabinoids activate transient receptor potential (TRP) channels. TRPs are cation channels activated by noxious heat or capsaicin (TRPV1 and 2) or cold and menthol (TRPA1). Activation by chemical ligands induces sensation, followed by rapid desensitization to produce antinociception (Akopian, Akopian, Ruparel, Patwardhan, & Hargreaves, 2008; Hagenacker, Ledwig, & Busselberg, 2008). TRPV1 is activated by anandamide and 2-AG, TRPV2 by THC and CBD, and TRPA1 by THC, CBD, and WIN55212-2 (Akopian et al., 2008; Kim, Cavanaugh, & Simkin, 2008; Movahed et al., 2005; Qin et al., 2008). CB1R and TRPV1 receptors are co-localized in cerebellum, basal ganglia, hippocampus, ventral PAG, diencephalon (Cristino et al., 2006) and DRG (Ahluwalia et al., 2000), and anandamide activation of TRPV1 inhibits the production and effects of 2-AG in striatum (Maccarrone et al., 2008). TRPV2 are found on the sensory neurons of the DRG, spinal cord, trigeminal ganglion and in cerebellum (Caterina, Rosen, Tominaga, Brake, & Julius, 1999; Kowase, Nakazato, Yoko, Morikawa, & Kojima, 2002; Qin et al., 2008). TRPA1 are often co-localized with TRPV1, and are found in sensory neurons and brain. Thus, some endocannabinoid effects might be produced via TRPV1, and exogenous cannabinoid effects via TRPV2 or TRPA1.

Cannabinoids activate two of the three nuclear peroxisome proliferator activated receptors (PPARs)

(O'Sullivan, 2007; Sun et al., 2007). PPARα is activated by anandamide and 2-AG, but not THC (Sun et al., 2007). PPARα activation reduces appetite, and elicits analgesia and neuroprotection. Anandamide, 2-AG, THC and CBD activate PPARγ, which has insulin-like effects on metabolism. PPARα and PPARγ are expressed in brain, peripheral nervous system, and peripheral tissues, especially those that regulate nutrient metabolism (Michalik et al., 2006). Moreover, activation of PPARγ and PPAR$\beta\gamma$ can affect activity of endocannabinoids and expression of CB1R and FAAH, suggesting a role in the endocannabinoid system (O'Sullivan, 2007). Some cannabinoid effects, such as analgesia and neuroprotection may be mediated by PPAR, TRP and CB1R, while other effects such as anti-inflammation might involve only PPARs. Further investigation is required to determine whether these additional G-protein-coupled, ion channel, and nuclear receptors are part of an integrated endocannabinoid system.

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